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Citation for published version:

Gilbert, N & Allan, J 2014, 'Supercoiling in DNA and chromatin', *Current Opinion in Genetics and Development*, vol. 25, no. 1, pp. 15-21. <https://doi.org/10.1016/j.gde.2013.10.013>

Digital Object Identifier (DOI):

[10.1016/j.gde.2013.10.013](https://doi.org/10.1016/j.gde.2013.10.013)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Current Opinion in Genetics and Development

Publisher Rights Statement:

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Supercoiling in DNA and chromatin[☆]

Nick Gilbert¹ and James Allan²

Supercoiling is a fundamental property of DNA and chromatin. It is modulated by polymerase and topoisomerase activities and, through regulated constraint, by DNA/chromatin binding proteins. As a non-covalent and elusive topological modification, supercoiling has proved intractable to research despite being a crucial regulator of nuclear structure and function. Recent studies have improved our understanding of the formation, regulation and organisation of supercoiling domains *in vivo*, and reinforce the prospect that the propagation of supercoiling can influence local and global chromatin structure. However, to further our understanding the development of new experimental tools and models are required to better dissect the mechanics of this key topological regulator.

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Current Opinion in Genetics & Development 2014, 25:15–21

This review comes from a themed issue on **Genome architecture and expression**

Edited by **Victor Corces and David L Levens**

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<http://dx.doi.org/10.1016/j.gde.2013.10.013>

Introduction

DNA is a dynamic molecule. In its relaxed state it adopts a right-handed helically coiled conformation, the detailed structure of which is dependent on the localised sequence. Winding DNA around its axis introduces supercoils increasing the free energy stored in the molecule; winding in the same direction as the helix introduces positive supercoiling whereas winding in the opposite direction generates negative supercoiling [1,2]. In addition to supercoiling derived from changes in DNA *twist*, it is also a product of the coiling or bending of the

helix in space, a parameter commonly termed *writhe*. *twist* and *writhe* are effectively inter-convertible and in a chromatin context it is useful to consider *writhe* as being of two types, constrained and unconstrained, the former resulting from direct physical wrapping around proteins or protein complexes. In prokaryotes, factors that package DNA, such as HU proteins, may control supercoiling by binding to DNA and trapping the free energy of supercoiling as *writhe* and subsequently releasing it through controlled dissociation [3,4]. Similarly in eukaryotes the regulated release of terminal DNA from a nucleosome, mediated by the acetylation of core histone tails, could release constrained *writhe* for conversion into negative supercoiling. Although *in vitro* studies support this concept [5] its operation *in vivo* is elusive [6].

In prokaryotes and eukaryotes all activities that require DNA to be unwound (and rewound) are potent generators of supercoiling. The classic example is the ‘twin supercoiled domain’ model where elongating RNA polymerase, in unwinding the DNA, generates positive supercoiling ahead and, in rewinding the DNA, generates negative supercoiling in its wake [7,8] (Figure 1). The levels of supercoiling produced in this process are prodigious, amounting to a positive and a negative supercoil for every 10 bp transcribed. Consequently the role of topoisomerases in releasing torsional stress is crucial if the template is to be maintained in a transcriptionally competent state. Genes that are negatively supercoiled are generally more efficiently transcribed [9,10] but topoisomerase inhibition studies [11,12^{*},13,14] indicate that the accumulation of excessive positive or negative supercoiling will repress transcription. Therefore, there must be a regulated balance in the localised levels of supercoiling through the concerted actions of polymerases [15] and topoisomerases [16,17].

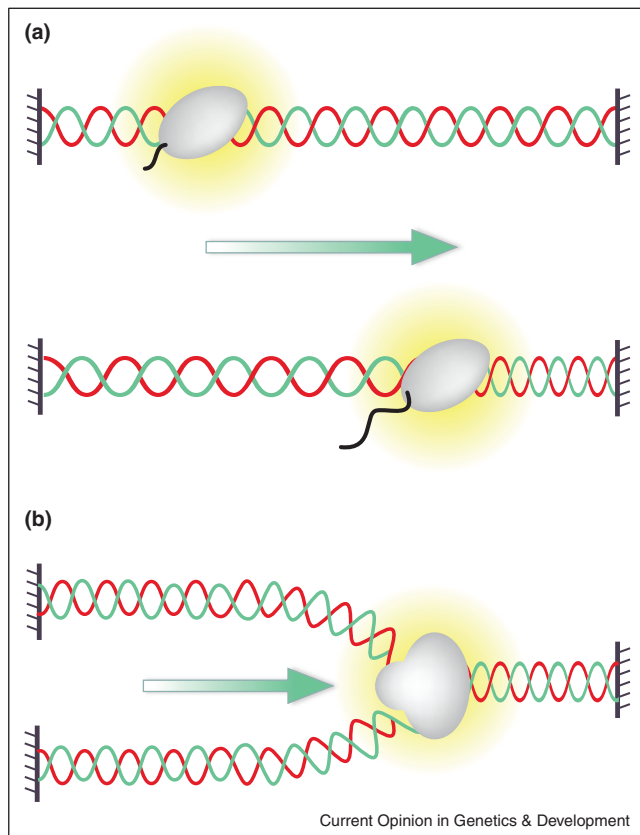
Supercoiling domains

When an activity supercoils DNA the torque generated is transmitted along the molecule. If the ends of the molecule are not fixed (or at least hindered), the supercoiling will dissipate via the unhindered rotation of the helix. Therefore for supercoiling to have a structural or functional influence on DNA or chromatin it must operate within a constrained environment where the energy is at least transiently trapped or restricted. For this reason it is anticipated that genomes need to be organised into supercoiling domains with barriers that prevent the spread of topological stress.

In prokaryotes the *Escherichia coli* genome has a hierarchical organisation based on large structural macrodomains

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Figure 1



(a) Twin domain model of DNA supercoiling. A transcribing polymerase generates positive supercoiling ahead and negative supercoiling behind. **(b)** Likewise, during replication the polymerase introduces positive supercoiling ahead whilst in contrast the newly replicated strands of DNA are in a relaxed configuration.

[3] with the Ter domain being subdivided into smaller, 35 kb domains via *MatS*/*MatP* interactions [18]. This organisation establishes a dynamic structural architecture enabling packaging without interfering with transcription or replication. The genome is also separately organised into about 500 independent ~10 kb supercoiling domains with demarcating barriers stochastically distributed and dynamically maintained [19,20]. However, as there are many more supercoiling domains than structural domains, the factors that define supercoiling boundaries must be distinct from those that characterise structural attachments.

Recent studies show that the eukaryotic genome is also organised into large (~1 Mb) loops, termed topologically associated domains (TADs) [21,22]. As these regions are invariant between cell types they appear to constitute a structural foundation to the genome and may not be directly relevant to functional activities such as transcription. The boundaries of TADs are enriched for CTCF binding sites. As some CTCF sites also recruit cohesion this suggests they may be involved in forming and

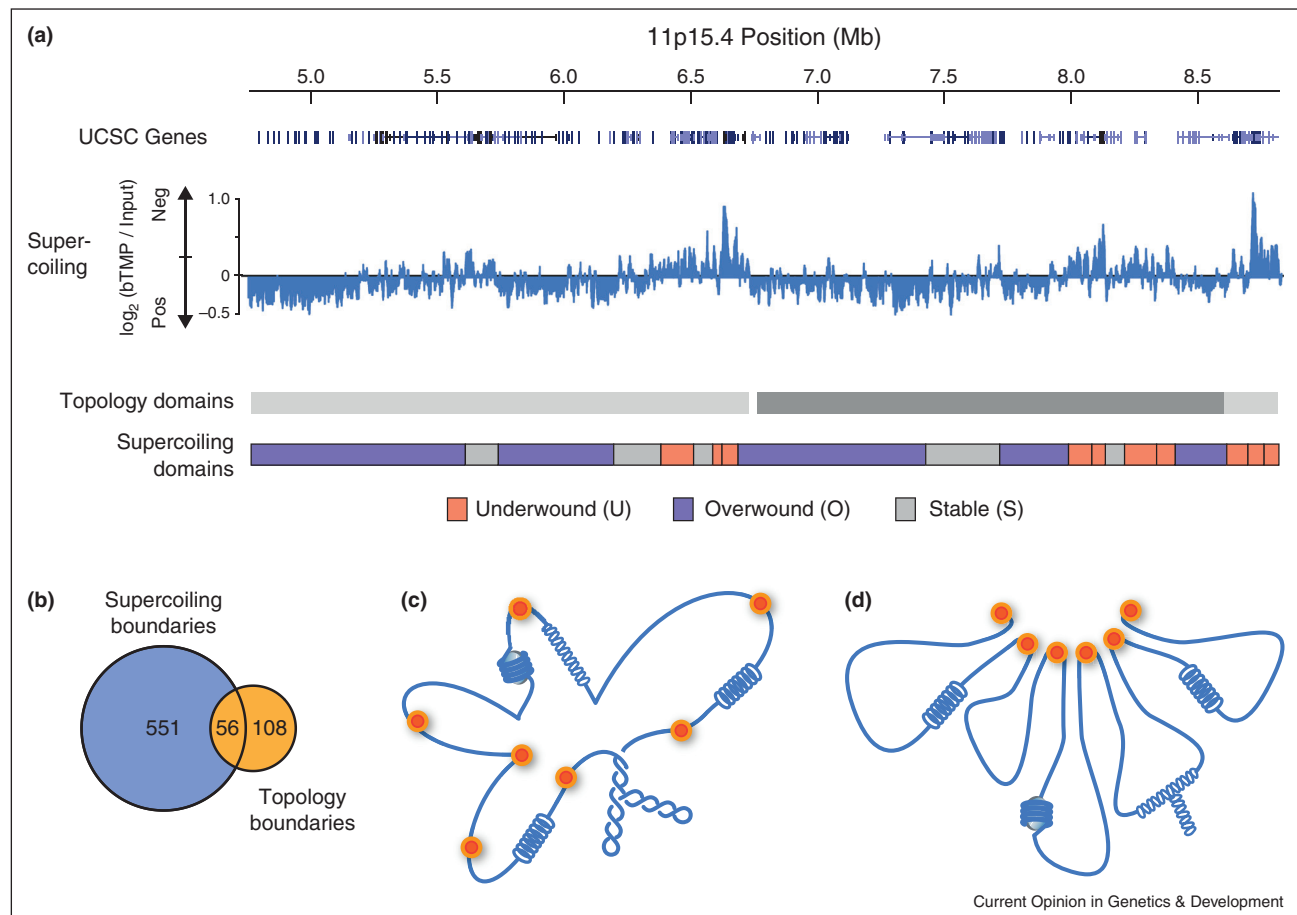
maintaining chromosomal loops and potentially act as supercoiling boundary elements.

To understand the nature of eukaryotic supercoiling domains, psoralen binding has been used in combination with microarrays to map the distribution of DNA supercoils across entire genomes [23] or to particular chromosomal regions [24^{••},25^{••}]. Psoralen preferentially intercalates into under-wound regions of the DNA helix and is fixed by long wave UV-light. To study supercoiling across large chromosomal domains in higher eukaryotes Naughton *et al.* [24^{••}] used a biotin-tagged psoralen molecule (bTMP) and mapped the distribution of drug binding using microarrays (Figure 2a). Analysis of human chromosome 11 revealed this DNA is divided into a series of relatively large (~100 kb) underwound and overwound domains. These domains were relaxed by bleomycin treatment (introduces DNA nicks) indicating they were, topologically, a dynamic genomic feature. Most strikingly, the patterns of these domains were transcription and topoisomerase dependent implying they were established by the competing activities of these enzymes. Approximately 10% of supercoiling domain boundaries coincided with TAD boundaries (Figure 2b) suggesting that some of these structural interaction nodes could be barriers to the passage of supercoils. However, as supercoiling domains are approximately one tenth the size of TADs the factors that define the majority of boundaries must be distinct from those that demarcate structural domains.

In a similar approach Kouzine *et al.* [25^{••}] also used psoralen to identify negatively supercoiled regions of the genome by isolating fragments of DNA resistant to denaturation due to psoralen cross-links. They focused on a subset of ENCODE promoters and showed that DNA supercoiling in these regions was restricted to relatively small foci (1.5 kb) centred upon transcription start sites. Supercoiling was dependent upon transcription with active genes being more negatively supercoiled than inactive genes. Inhibition of topoisomerases altered the pattern of DNA supercoiling and suggested that different topoisomerases might function separately on more highly and less highly transcribed genes.

Thus, as in bacteria, eukaryotic chromosomes appear to be organised into structural loops, overlaid with both large (100 kb) supercoiling domains and smaller supercoiling foci at transcription start sites (Figure 2c,d). The relationship between supercoiling domains and foci is not evident but domains may arise by supercoil diffusion from promoters. The mechanisms that constrain these domains are also unclear. Chromatin–chromatin interactions may act as supercoil diffusion barriers but the inherent drag, and therefore reduced rotation, caused by higher levels of chromatin organisation could in itself be sufficient to form the basis of supercoiling domains [26,27].

Figure 2



(a) Biotinylated-psoralen (bTMP) intercalates into the DNA helix preferentially at negatively supercoiled regions. bTMP binding, as a measure of DNA supercoiling, was mapped across a segment of chromosome 11 revealing the presence of underwound and overwound supercoiling domains (modified from Naughton *et al.* [24**]). **(b)** The sequences defining supercoiling domain boundaries and topology boundaries [22] partially overlap but indicate that different factors must be responsible. **(c)** Organisation of prokaryotic genomes into large structural domains with architectural boundaries (orange spots) but subdivided into smaller supercoiling domains. **(d)** Eukaryotic genomes are similarly organised into large structural domains delimited by CTCF rich boundaries (orange spots) and divided into many smaller supercoiling domains.

Effects of supercoiling on DNA

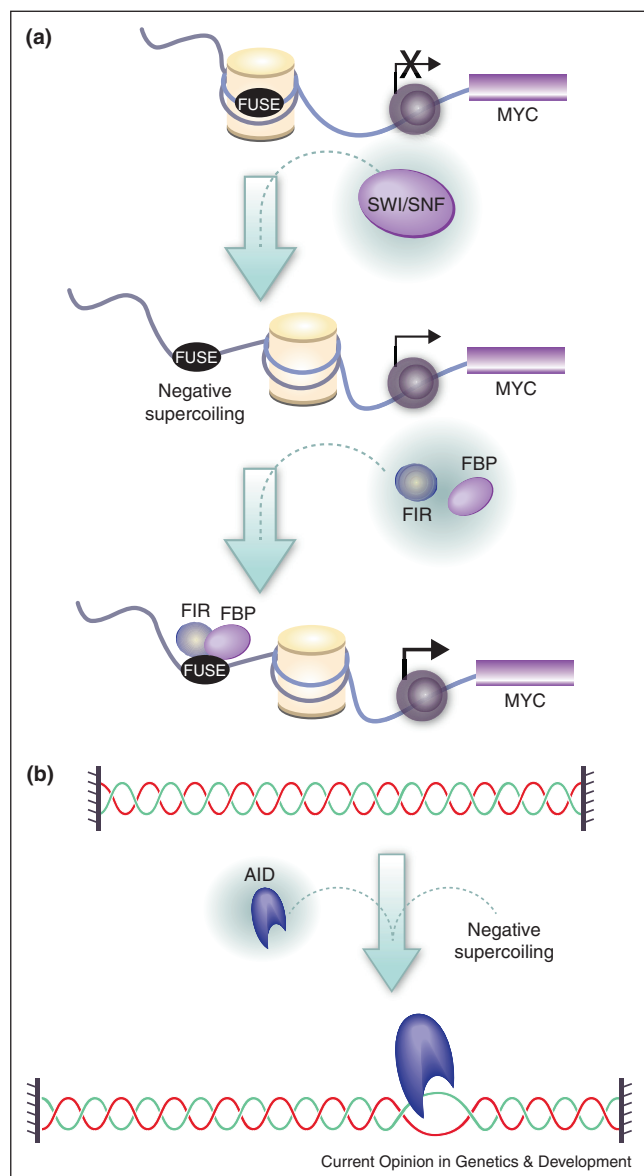
RNA polymerase generates about seven DNA supercoils per second. If these are not efficiently removed the residual energy may influence DNA or chromatin structure locally [28], or, if the energy can be propagated along the fibre, at more distant sites. The capacity of negative supercoiling to unwind DNA and facilitate processes such as transcription [29,30] and replication and its ability to induce alternative DNA structures such as cruciform [31], G-quadruplexes and Z-DNA [32] have been noted.

To address how transcription-generated force might directly alter DNA structure *in vivo*, Kouzine *et al.* [33] used a tamoxifen-inducible Cre recombinase to excise a chromatin segment with its torsional stress trapped intact. As the segment, flanked by loxP sites, had been positioned on a plasmid between divergently transcribing promoters it was demonstrated that as transcription

intensified the degree of negative supercoiling trapped within the excised segment increased. Using the c-myc FUSE element as a reporter they showed that supercoiling could propagate along the fibre, melt the FUSE element and promote the binding of ssDNA binding proteins (Figure 3a).

Although negative supercoiling promotes transcription initiation, supercoiling can also hinder polymerase elongation. To investigate how polymerase responds to different supercoiling environments Ma *et al.* [34**], in a single-molecule approach, used an angular optical trap. RNA polymerase was immobilised on a slide whilst its DNA template, attached to a quartz cylinder, was held in the trap. Rotation and torque could be applied to and measured from the DNA by manipulation of the quartz bead whilst its height provided a measure of displacement. Upon transcription into a negatively supercoiled

Figure 3



(a) Transcriptional activation of MYC is accompanied by a SWI/SNF activity revealing the FUSE element. A build up of negative supercoiling propagates along the fibre, melting the FUSE element promoting the binding of the ssDNA binding proteins, FBP and FIR [33,48]. **(b)** Negative supercoiling promotes ssDNA patch formation allowing the mutagenic activity of activation-induced cytidine deaminase (AID) [35*].

template, the polymerase initially relaxed the DNA and then introduced positive supercoiling. As positive supercoiling accumulated ahead of the polymerase, it stalled. Thus, resisting torque slows RNA polymerase and increases its pause frequency.

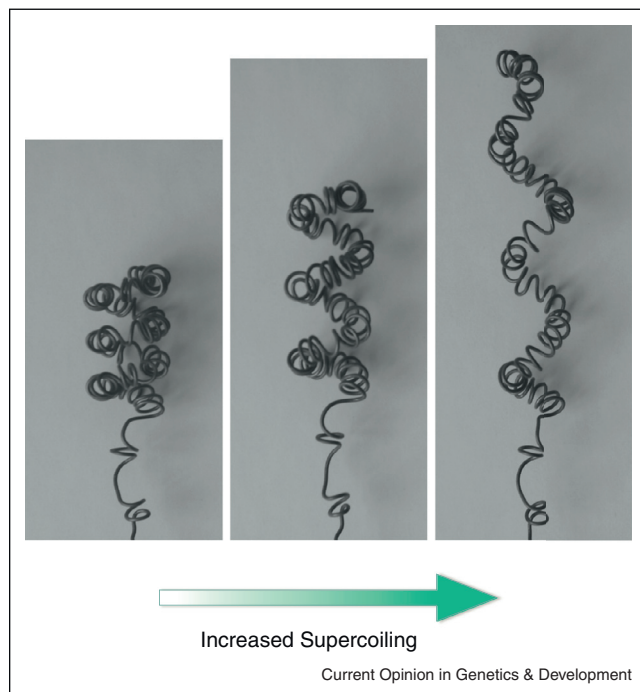
In addition to facilitating the binding of polymerases or transcription factors, negative supercoiling can generate DNA substrates for more complex activities. In yeast, topoisomerase I inhibition promotes the formation of

large ssDNA bubbles in highly expressed rRNA genes, which can be visualised by Miller spreads [12*]. Parsa *et al.* [35*] have shown that substantially smaller ssDNA patches located within active genes (Figure 3b) may be substrates for the mutagenic activity of activation-induced cytidine deaminase (AID). Employing an inducible gene in a hyper-negatively supercoiled *E. coli* strain they demonstrated that negative supercoiling increased ssDNA patch density compared to wild type and promoted a higher mutation rate. It will be interesting to know whether a similar effect is observed in eukaryotic cells where the DNA is packaged into chromatin and levels of supercoiling are probably buffered.

Effects of supercoiling on chromatin

In eukaryotic cells the effects of supercoiling have to be considered in the context of chromatin but unfortunately, we know very little about this situation. At the level of the 'twin supercoil domain' the scenario seems simplistic; positive supercoiling ahead of the polymerase will destabilize nucleosome structure and negative supercoiling behind will promote reassembly [36], actions that seem entirely consistent with the thermodynamic demands of transcription through a chromatin fibre. However, the many models that purport to explain the mechanics of how polymerase does in fact transcribe through a nucleosome reflects our ignorance of the details [37]. Things are no clearer at higher levels of chromatin structure. The idea that supercoiling might be generated at one site, say at a transcriptionally active gene, and then transmitted through the chromatin fibre to another location to create or remodel a domain or to influence a distant process, hinges on the concept that torsion can be transmitted along the fibre (Figure 4). Although we raised this issue, twenty-five years ago [38], the question essentially remains unanswered as the difficulty is multifaceted. We do not have a good understanding of the structure(s) that the higher-order chromatin fibre adopts, and yet this will undoubtedly constitute a profound influence upon the ability to transmit supercoiling. In addition, the composition and modification of the components of the fibre are also likely to affect its plasticity. Nucleosomes containing yeast histones are more sensitive to thermally induced torsional stress [39] than nucleosomes containing higher eukaryotic core histones suggesting, perhaps, a greater propensity for yeast chromatin to absorb rather than transmit negative supercoiling. In spite of these reservations pioneering single-molecule studies have attempted to provide an insight into this fundamental question. Using magnetic tweezers to introduce torsional stress into model chromatin fibres Bancaud *et al.* [40] found chromatin to be highly accommodating of supercoiling. To illustrate, they argued that supercoiling generated by transcribing 100 bp of DNA could be absorbed within a 10 kb chromatin fragment thereby diminishing the need for topoisomerase relaxation. Although such plasticity may not be typical of more condensed, native

Figure 4



Model representing how changes in supercoiling could influence the structure of the 30-nm chromatin fibre. Here twist is shown to cause the fibre to over-wind or under-wind altering its tertiary conformation and potentially enabling the torsional stress to be propagated to distant sites.

chromatin fibres, it does provide insight into the buffering capacity of chromatin to supercoiling and its transmission.

Although there is no direct evidence for the transmission of torsion through chromatin the phenomenon is used to explain the apparent dissipation of positive supercoiling near the ends of yeast chromosomes [41]. The rotation of the terminal 100 kb of the chromosome is argued to be the means of releasing positive supercoiling, in spite of telomere attachment and substantial rotational drag [26]. In a related study Kegel *et al.* [42] observed that inhibition of topoisomerase I and the build up of positive supercoiling caused replication delay in long but not short yeast chromosomes. From this they suggested that supercoiling stress was more problematic for large chromosomes where its dissipation was less easily achieved through chromosome rotation.

DNA supercoiling also has a major role during DNA replication and the subsequent condensation and separation of replicated chromosomes. Positive supercoiling, generated in front of the DNA polymerase during replication (Figure 1b), is relaxed by topoisomerases I and II. However, when converging forks approach, relaxation of positive supercoiling is restricted and the build up of torsional stress causes swivelling of the replication complex required to complete replication [43•]. This causes intertwining of newly replicated DNA molecules behind

the fork and the formation of precatenanes. Subsequently, most but not all catenanes are removed by topoisomerases II. On approaching mitosis the remaining catenations, or sister chromatid intertwinings are 'identified' by a process that involves an architectural change in chromatin structure, orchestrated by condensin-generated and mitotic spindle-dependant positive supercoiling [44]. This structural change then allows topoisomerase II to identify and resolve inter-chromosomal but not intra-chromosomal crossovers. Concomitantly, chromosome compaction starts during S-phase when condensin II is recruited to replicated regions [45]. Condensins introduce global positive writhe into the DNA/chromatin *in vitro* [46] and as a result changes in supercoiling energy are thought to co-dependently drive mitotic chromosome architecture [47] and resolution *in vivo*. Understanding how these processes are linked and determine the cytological chromosome structure will be key areas of future research.

Conclusions

A renewed interest in supercoiling research is clarifying how it influences nuclear processes and architecture. However, a lack of fundamental knowledge of the multi-layered structures of its substrate, the chromatin fibre, and given that supercoiling is such an inherently elusive topological force, will probably demand the development of new and innovative experimental approaches. The development of topologically constrained models of physiologically relevant chromatin fibres will enable studies of fibre stability, interplay between polymerases and topoisomerases and the propagation of supercoiling energy. Whilst minimally invasive probes are necessary to analyse chromatin structure and the distribution of supercoiling *in vivo*. With new approaches these will be exciting times for this area of research.

Acknowledgements

We would like to thank Sam Corless for crucial comments on the manuscript and Jon Baxter for his helpful insights into DNA supercoiling in transcription and replication. Research described in this review was supported by the Wellcome Trust and NG is now funded by the UK Medical Research Council.

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